Thymidine Kinase Activity in Burkitt Lymphoblastoid Somatic Cell Hybrids After Induction of the EB Virus[1] (37176)

R. GLASER, T. OGINO, J. ZIMMERMAN, JR., AND F. RAPP

Department of Microbiology, College of Medicine, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, Pennsylvania 17033

Introduction. It has recently been shown that Burkitt lymphoblastoid cells can be hybridized with both mouse and human cells, using inactivated Sendai virus (1). The Epstein–Barr virus (EBV) was induced in the somatic cell hybrids, which had been negative for EBV markers, following exposure of the cells to 5-iododeoxyuridine (IUDR) (2). Since deoxythymidine kinase (TdR kinase) activity increases in cultured cells after infection with a variety of viruses (3), and since participation of the virus genome in coding for TdR kinase has been suggested for herpes simplex virus (4, 5), we undertook the present study to determine the activity of TdR kinase in Burkitt lymphoblastoid somatic cell hybrids after treatment with IUDR.

Materials and Methods. Cells. The cell lines used in the study have been previously described (1, 2). The human cell line D98/AH-2 (D98) was maintained in Eagle's medium. The Burkitt lymphoblastoid cell line P3J-HR-1 (HR-1) was maintained in RPMI 1640 medium. The hybrid cell line (D98/HR-1), clones (cl) 1 and 8 (2) were maintained in HAT medium (1, 2). All media used contained 10% fetal calf serum, 0.075% $NaHCO_3$, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 1 μg/ml of fungizone and 10 units/ml of mycostatin.

Induction. To induce EBV, the hybrid cells were incubated in Eagle's medium containing 60 μg/ml of IUDR at 37° for three days. At that time, the Eagle's medium containing the drug was replaced with normal Eagle's medium and the cultures harvested for assay of enzymes on Days 1, 2, 3, and 7. In these experiments, the day of removal of IUDR was taken as Day 0.

TdR kinase assay. All cells (attached on glass and floating cells) were pooled for the assay of TdR kinase activity. Cells were scraped off the 8 oz glass bottles or plastic tissue culture flasks with a rubber policeman into medium and centrifuged at low speed. Cell pellets were washed once with Tris (hydroxymethyl) aminomethane (Tris) buffered saline (0.025 *M*, pH 7.4). Cell pellets were resuspended in 0.05 *M* Tris buffer (pH 8.0) containing 0.15 *M* KCl, 3 m*M* 2-mercaptoethanol and subjected to ultrasonic disintegration. The extracts were centrifuged at 15,000 rpm for 60 min in an SS-34 rotor in a Sorvall RC2-B centrifuge. The supernatant fractions were used as the source of enzyme. TdR kinase activity was assayed by the DEAE-cellulose paper disc method using the following reaction mixture: ^{14}C TdR, 0.1–0.2 μCi (35 mCi/mmole), 5 m*M* ATP, 5 m*M* $MgCl_2$, cell extracts and 0.05 *M* Tris buffer (pH 8.0) up to 0.25 ml (6). Protein determinations were carried out by the method of Lowry *et al.* (7) using bovine serum albumin as the reference standard.

Deoxycytidine (dCMP) deaminase assay. For the assay of dCMP deaminase, 5×10^6 D98/HR-1 hybrid cells were homogenized in a Dounce homogenizer in 1 ml of 0.02 *M* Tris buffer, pH 7.4, containing 2.0 m*M* 2-mercaptoethanol, 1.5 m*M* $MgCl_2$, and 0.3 m*M* dCTP as an enzyme stabilizer. The homogenate was then frozen in an acetone–dry ice bath and thawed. The homogenate was centrifuged for 30 min at 15,000*g* and the activity of the enzyme was determined in the

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supernatant fluid as described by Gelbard *et al.* (8), except that deoxycytidylate and deoxyuridylate were separated by chromatography on Whatman No. 1 paper with isobutyric acid–water–15 *N* ammonia (66:33:1.5). After drying, chromatographs were cut into strips and the strips were cut to 1 mm wide fractions which were counted in a Beckman LS233 liquid scintillation counter using an omnifluor-toluene cocktail. Protein determination was by the method previously described (7).

Results. TdR kinase induction. When D98/HR-1 cl 1 cells were assayed for TdR kinase activity after induction of EBV with IUDR, it was found that a corresponding increase in TdR kinase activity could be demonstrated (Fig. 1). This increase was reproducible and generally declined to the control level within 6–7 days after removal of the IUDR. The TdR kinase activity of the drug treated hybrid cells, as well as both parental cells, D98 and HR-1, was then assayed on Day 1, 2, 3, and 7 after removal of IUDR. The results are summarized in Table I. TdR kinase activity in the D98/HR-1 cl 8 cells

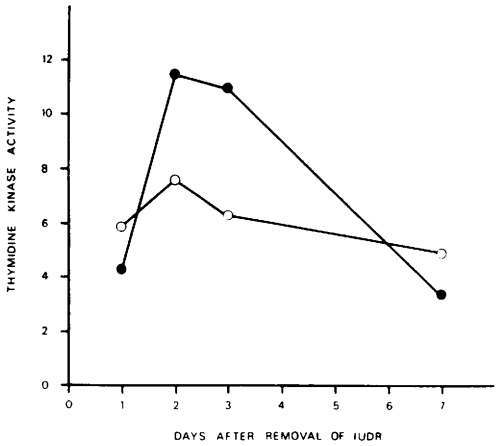

FIG. 1. Activity of TdR kinase in control and IUDR treated D98/HR-1 cl 1 hybrid cells. D98/HR-1 cl 1 cells were treated with 60 μg/ml of IUDR for three days. IUDR supplemented medium was removed, fresh medium added and cells were harvested at the times indicated. TdR kinase was assayed in cell free extracts as described in Materials and Methods. TdR kinase activity is represented as mμmoles of phosphorylated TdR/mg protein/15 min at 38°. (●) IUDR treated D98/HR-1 cl 1 hybrid cells; (○) control D98/HR-1 cl 1 hybrid cells.

TABLE I. TdR Kinase Activity[a] in Somatic Cell Hybrids of Burkitt Lymphoblastoid and Human Cells.

	Cell assayed		
Time of assay	D98	D98/HR-1 cl 8	HR-1
Day 1[b]	3.2	5.7	9.7
Day 2	5.2	9.4	10.8
Day 3	3.4	10.5	12.3
Day 7	4.2	5.1	4.8

[a] TdR kinase activity is shown as mμmoles [^{14}C] TdR phosphorylated/mg protein/15 min at 38°.

[b] Cells were treated with IUDR (60 μg/ml) at 37° for 3 days, at which time medium with IUDR was replaced by medium without IUDR. "Day 1" means one day after removal of IUDR.

increased after treatment with IUDR, peaking in activity between Days 2 and 3. TdR kinase activity in D98 cells, which do not contain the EBV genome (9) did not increase following exposure to IUDR. When HR-1 cells, which do contain EBV genome (10, 11), were assayed in the same manner, the activity of TdR kinase increased and the pattern of increase was similar to that obtained with the D98/HR-1 cells. Thus, the two cell lines containing the EBV genome showed increase in TdR kinase activity following exposure to IUDR.

dCMP deaminase activity. D98/HR-1 cl 1 and 8 cell cultures were assayed for dCMP deaminase activity after treatment of the cell cultures with 60 μg/ml IUDR for three days. No significant increase in dCMP deaminase occurred following treatment of the D98/HR-1 cl 1 cells with IUDR (Fig. 2). A similar result was obtained for D98/HR-1 cl 8. The slight increase in activity which occurs in both IUDR and control cultures at Day 2 may be a result of nutrient replenishment. The same effect may account for the slight increase of TdR kinase activity on Day 2 in the D98 cells (Table I) and on Day 2 in control (nondrug treated) D98/HR-1 cl 1 cells (Fig. 1). The greater decrease in activity of dCMP deaminase in IUDR treated cells is presumably a result of cell death in those cells which were induced.

Discussion. The *de novo* cellular biosynthetic pathway leading to the provision of deoxythymidine triphosphate for DNA syn-

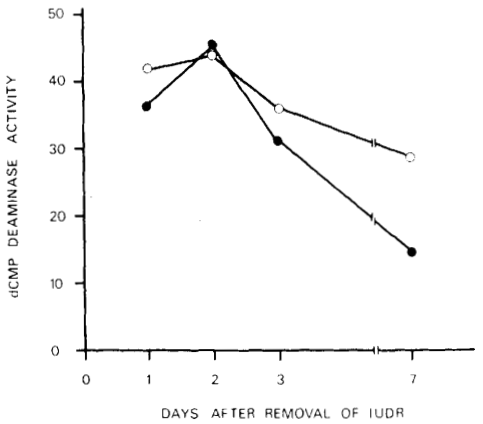

FIG. 2. Activity of dCMP deaminase in control and IUDR treated D98/HR-1 cl 1 hybrid cells. D98/HR-1 cl 1 cells were treated with 60 μg/ml IUDR for three days, IUDR supplemented medium was removed, fresh medium added, and cells were harvested at the times indicated. dCMP deaminase was assayed in cell free extracts as described in Materials and Methods. dCMP deaminase activity is represented as nmoles of dUMP formed/mg protein/15 min at 37°. (●) IUDR treated D98/HR-1 cl 1 hybrid cells; (○) control D98/HR-1 cl 1 hybrid cells.

thesis involves the processing of cytidine diphosphate through several steps to deoxythymidine monophosphate (CDP → dCDP → dCMP → dUMP → dTMP). In the normal cell, TdR kinase is involved as a "scavenger" enzyme and is not used in the primary pathway of the DNA synthesizing apparatus. The induction of TdR kinase activity might be virus coded or the increase in activity could be coincidental with a general induction of host enzymes involved in DNA synthesis. In order to see if a general induction of activity occurred, the activity of (dCMP) deaminase was assayed. This enzyme is responsible for the deamination of deoxycytidylic acid to deoxyuridylic acid and is central in the cellular pathway for the processing of deoxythymidylate from cytidine diphosphate.

It has been demonstrated that D98/HR-1 cells, under normal growth conditions, do not express EBV markers (1, 2). When four clones of the hybrid cells were examined for the presence of EBV DNA by nucleic acid hybridization, all were found to contain EBV DNA (9). When these cells were treated with IUDR, EBV was induced, presumably due to the activation of EBV DNA followed by synthesis of virus antigens and particles. A corresponding increase in hybridizable EBV DNA was also observed (9). The highest level of antigen expression, as determined by the immunofluorescence test, was obtained three days after removal of IUDR and EBV particles could be detected four days later (7 days after removal of IUDR) by electron microscopy (2).

The pattern of increase in TdR kinase activity of D98/HR-1 cells shown in this study is coincident with the patterns of appearance of virus antigens and virus particles described above and also corresponds closely to the activity observed in HR-1 cells treated in the same way. The increased TdR kinase activity in the IUDR treated hybrid cells with the corresponding appearance of antigen positive cells and an increase in hybridizable EBV DNA suggest that the induction of TdR kinase may be related to the reactivation of the EBV genome. A similar increase in TdR kinase has recently been reported after the induction of EBV from 5-bromodeoxyuridine (BUDR) resistant HR-1 cells which are TdR kinase negative (12). In addition, the thermal denaturation characteristic of the induced TdR kinase was found to be very similar to cellular TdR kinase. This has also been found in the BUDR resistant HR-1 induction system (Dr. Berge Hampar, personal communication). In the case of herpes simplex virus (HSV), there is good evidence that HSV can direct synthesis of TdR kinase during lytic infection (4, 5) and can transform TdR kinase negative cells into TdR kinase positive cells (13).

Expression of the virus genome of polyoma, SV40 (3), and adenovirus (3, 14) resulted in elevated levels of activity of all the enzymes involved in the processing of cytidine 5′ diphosphate to deoxythymidine 5′ monophosphate as well as TdR kinase and DNA polymerase. The information for this general induction resides in the virus genome (3). TdR kinase negative cells did not acquire TdR kinase activity after infection (3, 15) and the number of early virus genes transcribed precludes the possibility that the virus coded for all these functions. However, the size of the

EBV genome is not limiting, TdR kinase activity is stimulated, and at least one of the enzymes (dCMP deaminase) centrally involved in the processing of dTMP is not increased in activity when the EBV genome is expressed. The mechanism by which TdR kinase activity is increased in the process of virus activation may represent another virus genome function that is derepressed. If TdR kinase is not virus coded, some virus gene product would have to selectively derepress certain host enzymes involved in DNA synthesis or selectively stimulate pre-existing host enzyme activity.

Summary. Epstein–Barr virus (EBV) can be rescued from somatic cell hybrids after treatment with 5-iododeoxyuridine (IUDR). Deoxythymidine kinase activity increased in the hybrid cells after induction of EBV. Activity of deoxycytidine deaminase did not increase significantly following induction of the virus. The evidence suggests that the EBV genome may participate in the increased enzyme activity observed.

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